

WHAT IS CLAIMED IS:

1. An isolated polynucleotide comprising:

a first nucleotide sequence comprising the coding region for a first leader peptide,

wherein said first leader peptide comprises

(1) two or more positively charged amino acids close to the N-terminus,

(2) a region of between 7 and 16 consecutive hydrophobic amino acid residues, and

(3) at the C-terminus, the sequence Z-X-Z, wherein each Z is independently an amino acid having a small side chain and X is any genetically encoded amino acid; and

a second nucleotide sequence comprising a first ribosome binding site, wherein said second nucleotide sequence is 5' of said first nucleotide sequence, wherein said first ribosome binding site is operatively joined to said coding region for said first leader peptide, and wherein, when said polynucleotide is RNA or is transcribed into RNA, said first ribosome binding site is accessible.

2. The polynucleotide of claim 1, wherein said first leader peptide further comprises an amino acid which acts as an alpha helix disrupter, wherein said alpha helix disrupter amino acid is located between said region of consecutive hydrophobic amino acid residues and said Z-X-Z sequence.

3. The polynucleotide of claim 1 or claim 2, further comprising a third nucleotide sequence comprising the coding region for a first recombinant protein, wherein said third nucleotide sequence is 3' of said first nucleotide sequence and is operatively joined to said first nucleotide sequence in such manner that a first fusion polypeptide comprising said first leader peptide joined to said first recombinant protein is encoded.

4. The polynucleotide of claim 3, wherein said first recombinant protein is a human growth hormone, an interferon, an immunoglobulin, insulin, or an immunoadhesin.

5. The polynucleotide of claim 3, wherein said first recombinant protein is an immunoglobulin light chain, an immunoglobulin heavy chain, an immunoglobulin light chain or heavy chain fragment, or a scFv.

6. The polynucleotide of claim 3, further comprising a fourth nucleotide sequence and a fifth nucleotide sequence, wherein said fourth nucleotide sequence is 3' of said third nucleotide sequence, and said fifth nucleotide sequence is 3' of said fourth nucleotide sequence, wherein said fourth nucleotide sequence comprises the coding region for a second leader peptide, wherein said second leader peptide comprises (1) two or more positively charged amino acids close to the N-terminus, (2) a region of between 7 and 16 consecutive hydrophobic amino acid residues, and (3) at the C-terminus, the sequence Z-X-Z, wherein each Z is independently an amino acid having a small side chain and X is any genetically encoded amino acid, and said fifth nucleotide sequence comprises the coding region for a second recombinant protein, wherein said fourth nucleotide sequence is operatively joined to said fifth nucleotide sequence in such manner that a second fusion polypeptide comprising said second leader peptide joined to said second recombinant protein is encoded, wherein the coding region for said second leader peptide is separated from the coding region for said first recombinant protein by between 1 and 30 nucleotides,

and wherein, when said polynucleotide is RNA or is transcribed into RNA, said first ribosome binding site is accessible.

7. The polynucleotide of claim 6, wherein said second leader peptide further comprises an amino acid which acts as an alpha helix disrupter, wherein said alpha helix disrupter amino acid is located between said region of consecutive hydrophobic amino acid residues and said Z-X-Z sequence

8. The polynucleotide of claim 6, wherein said first recombinant protein and said second recombinant protein are polypeptide subunits of a multimeric protein.

9. The polynucleotide of claim 6, wherein said first recombinant protein and said second recombinant protein are independently selected from the group consisting of an

immunoglobulin light chain, an immunoglobulin heavy chain, an immunoglobulin light chain fragment or an immunoglobulin heavy chain fragment.

10. An isolated polynucleotide comprising

5 a first nucleotide sequence comprising the coding region for a first leader peptide having the amino acid sequence $M-X_n-(K/R)-(K/R)-J_m-P-X_p-Z-X-Z$, wherein M is methionine, K is lysine, R is arginine, (K/R) represents either lysine or arginine, P is proline, each X is independently any genetically encoded amino acid, each J is independently an amino acid selected from the group consisting of alanine, leucine,
10 valine, phenylalanine, threonine, isoleucine, serine, glutamine, asparagine, methionine, and tyrosine, each Z is independently an amino acid selected from the group consisting of alanine, serine, glycine, valine and threonine, n is an integer from 1 to 2, p is an integer from 0 to 2, and m is an integer from 7 to 16; and

a second nucleotide sequence comprising a first ribosome binding site, wherein said
5 second nucleotide sequence is 5' of said first nucleotide sequence, wherein said first ribosome binding site is operatively joined to said coding region for said first leader peptide, and wherein, when said polynucleotide is RNA or is transcribed into RNA, said first ribosome binding site is accessible.

11. An isolated polynucleotide comprising a first nucleotide sequence comprising a
coding region for a first leader peptide having the amino acid sequence selected from the group
consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO: 3, SEQ ID NO:4 and SEQ ID NO:23;
and

a second nucleotide sequence comprising a first ribosome binding site wherein said
25 second nucleotide sequence is 5' of said first nucleotide sequence, wherein said first ribosome binding site is operatively joined to said coding region for said first leader peptide, and wherein, when said polynucleotide is RNA or is transcribed into RNA, said first ribosome binding site is accessible.

30 12. The polynucleotide of claim 10 or claim 11, further comprising a third nucleotide sequence comprising the coding region for a first recombinant protein, wherein said third

nucleotide sequence is 3' of said first nucleotide sequence and is operatively joined to said first nucleotide sequence in such manner that a first fusion polypeptide comprising said first leader peptide joined to said first recombinant protein is encoded.

13. The polynucleotide of claim 11, comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

14. An expression vector comprising the polynucleotide of claim 1 and a promoter, wherein said promoter is located 5' of and operatively joined to said second nucleotide sequence, whereby the transcription of said first nucleotide sequence is controlled by said promoter.

15. An expression vector comprising the polynucleotide of claim 3 and a promoter, wherein said promoter is located 5' of and operatively joined to said second nucleotide sequence, whereby the transcription of said first nucleotide sequence and said third nucleotide sequence is controlled by said promoter, resulting in the production of an mRNA encoding said first fusion polypeptide.

16. An expression vector comprising the polynucleotide of claim 6 and a promoter, wherein said promoter is located 5' of and operatively joined to said second nucleotide sequence, whereby the transcription of said first nucleotide sequence and said third nucleotide sequence and said fourth nucleotide sequence and said fifth nucleotide sequence is controlled by said promoter, resulting in the production of an mRNA encoding said first fusion polypeptide and said second fusion polypeptide.

17. The expression vector of claim 16, wherein said first recombinant protein and said second recombinant protein are polypeptide subunits of a multimeric protein.

18. The expression vector of claim 16, wherein said first recombinant protein and said second recombinant protein are independently selected from the group consisting of an immunoglobulin light chain, an immunoglobulin heavy chain, an immunoglobulin light chain fragment or an immunoglobulin heavy chain fragment.

19. The expression vector of claim 14 wherein said promoter is a bacterial promoter.

20. The expression vector of claim 19, wherein said promoter is selected from the group consisting of the lac promoter, the trp promoter, the ara promoter, the beta-lactamase promoter and the lambda P_L promoter.

21. A method for producing a recombinant protein in a host cell comprising transforming a host cell with the expression vector of claim 15, wherein said promoter is functional in said host cell, culturing said host cell under conditions such that said first fusion polypeptide is expressed and secreted from said host cell, and isolating said first recombinant protein.

22. The method of claim 21, wherein said host cell is a bacterial cell.

23. A method for producing a recombinant protein in a host cell comprising transforming a host cell with the expression vector of claim 16, wherein said promoter is functional in said host cell, culturing said host cell under conditions such that said first fusion polypeptide and said second fusion polypeptide are expressed and secreted from said host cell, and isolating said first recombinant protein and said second recombinant protein.

24. The method of claim 23, wherein said host cell is a bacterial cell.

25. A fusion polypeptide comprising a leader peptide joined to a recombinant protein, such that the carboxy terminus of the leader peptide is joined to the amino terminus of the recombinant protein, wherein the leader peptide comprises (1) two or more positively charged amino acids close to the N-terminus, (2) a region of between 7 and 16 consecutive hydrophobic amino acid residues, and (3) at the C-

terminus, the sequence Z-X-Z, wherein each Z is independently an amino acid having a small side chain and X is any genetically encoded amino acid.

26. The fusion polypeptide of claim 25, wherein said leader peptide further comprises an amino acid which acts as an alpha helix disrupter, wherein said alpha helix disrupter amino acid is located between said region of consecutive hydrophobic amino acid residues and said Z-X-Z sequence.

27. The fusion polypeptide of claim 25, wherein said leader peptide has an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 and SEQ ID NO:23.

28. A method for designing a polynucleotide encoding a fusion polypeptide for enhanced secretion of the fusion polypeptide comprising:

(a) selecting a first nucleotide sequence comprising the coding region for a leader peptide, wherein said leader peptide comprises

- (1) two or more positively charged amino acids close to the N-terminus,
- (2) a region of between 7 and 16 consecutive hydrophobic amino acid residues, and

(3) at the C-terminus, the sequence Z-X-Z, wherein each Z is independently an amino acid having a small side chain and X is any genetically encoded amino acid;

(b) selecting a second nucleotide sequence comprising a ribosome binding site, wherein said second nucleotide sequence is joined to said first nucleotide sequence such that said second nucleotide sequence is 5' of said first nucleotide sequence, and wherein when said joined first and second nucleotide sequence is RNA or is transcribed into RNA, said ribosome binding site is accessible;

(c) selecting a third nucleotide sequence encoding a recombinant protein, wherein said third nucleotide sequence is joined to said first nucleotide sequence in such manner that a fusion polypeptide comprising said leader peptide joined to said recombinant protein is encoded; and

(d) assembling said selected first, second and third nucleotide sequences into a single polynucleotide.

29. The method of claim 28, wherein said leader peptide further comprises an amino acid which acts as an alpha helix disrupter, wherein said alpha helix disrupter amino acid is located between said region of consecutive hydrophobic amino acid residues and said Z-X-Z sequence.

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